3784-Pos
Growth Process and Mechanics of Cell Adhesion Investigated by Optical Tweezers
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Cell adhesion between extracellular matrix and integrin is one of essential structure for cell growth, differentiation and cell motility. Paxillin and vinculin reinforce strength of cell adhesion and Src controls aggregation of these proteins at initial phase of cell adhesion. Thus the relationship between molecular and physical properties is important to understand the cell adhesion mechanisms. However, we still don't fully understand the property of cell adhesion. To investigate the characteristics, we have developed a measurement system for the stiffness of cell adhesion by using a collagen coated particle (2-micron diameter), which is manipulated by optical tweezers. In this system, cell adhesion is created under the particle by attaching to mouse fibroblast (Balb-3T3) for 1 min and it is called ''initial adhesion''. After that the particle is moved back-and-forth in sinusoidal manner and analyzed the position of the particle recorded by a CCD camera. From that data we can know the actual force exerted on the particle. The minimum force sensitivity of the system is 80 fN. By using this system, we are able to measure the force of cell adhesion during growth process. This force rapidly increases for 30min from initial adhesion and gradually increases after 30 min. And we investigate the force change for 30 seconds after creation of initial adhesion. As a result, we observe the growth of cell adhesion with reinforcement and relaxation of adhesion force. And the stabilization of the reinforcement is occurred with fluctuation of the adhesion force. These phenomena are basic properties of cell adhesion and are important to reveal of molecular mechanism of cell adhesion.

3785-Pos
H2-Calponin Regulates Adhesion and Migration of Macrophages
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Calponin is an actin filament-associated regulatory protein. The h2 isoform of calponin is found in both smooth muscle and non-muscle cells including monocytes and macrophages. We previously demonstrated that h2-calponin plays an inhibitory role in the regulation of cell proliferation and migration and increases the stability of actin cytoskeleton (Hossain et al., *AJP*: 284:C156-67, 2003; *JBC* 280:42442-53, 2005; *Biochemistry* 45:15670-83, 2006). Using residential cells isolated from the intraperitoneal cavity of h2-calponin knockout mice (Huang et al., *JBC* 283:25887-99, 2008), the present study investigated the role of h2-calponin in macrophage motility. Substrate adhesion of h2-calponin-null macrophages was significantly decreased together with reduced cell spreading area in culture dish. The h2-calponin deficient macrophages further exhibited increased migration and transendothelial migration. The deficiency of h2-calponin did not affect macrophage invasion into Matrigel, suggesting that the increased transendothelial migration is based on unchanged activity of extracellular proteases. H2-calponin was co-localized with F-actin in thin spikes at all edges of stationary macrophages and the trailing edges of migrating cells while absent in the leading edge lamellipodium. Consistent with increased motility, h2-calponin-null macrophages exhibited losses of these anchoring spikes. The results suggest a role of h2-calponin in inhibiting macrophage motility and transendothelial migration through stabilization of actin cytoskeleton.

3786-Pos
Nucleation and Growth of Integrin Adhesions
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We present a model that provides a mechanistic understanding of the processes that govern the formation of the earliest integrin adhesions ex novo from an approximately planar plasma membrane. Using an analytic analysis of the free energy of a dynamically deformable membrane containing freely diffusing receptors molecules and long repeller molecules that inhibit integrins from binding with ligands on the extracellular matrix, we predict that a coalescence of polymerizing actin filaments can deform the membrane toward the extracellular matrix and facilitate integrin binding. Monte Carlo simulations of this system show that thermally induced membrane fluctuations can either zip-up and increase the radius of a nucleated adhesion or unzip and shrink an adhesion, but the fluctuations cannot bend the ventral membrane to nucleate an adhesion. To distinguish this integrin adhesion from more mature adhesions, we refer to this early adhesion as a nouveau adhesion.

3787-Pos
Adipogenic Commitment of Mesenchymal Stem Cells Regulated by ERM Proteins-Mediated Cellular Biomechanics
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Cellular mechanics plays an important role in many cell activities including, to name just a few, morphogenesis, migration, proliferation, and differentiation. Adipogenic differentiation of human mesenchymal stem cells (hMSC) is found to cause a decrease both in the cytoskeleton elasticity and membrane-cytoskeleton association and is mediated by the ERM (ezrin, radixin, moesin) family of protein linkers. Transient knockdown of ERM proteins with RNAi technique results in membrane separation from the cytoskeleton in hMSC as determined using optically extracted membrane tethers. In addition, it leads to a substantial decrease in the cell elasticity measured using AFM microindentation. This cytoskeleton biomechanics modulation is likely mediated by a partial disassembly of actin stress fibers and focal adhesions during ERM linkers knockdown. Although this kind of treatment induces changes in the stem cell mechanical properties similar to those of fully differentiated adipocytes, hMSC commitment by soluble adipogenic factors is impaired in the ERM-deficient cells. However, cell mechanics modulation by ERM knockdown following a 6-day adipogenic induction by soluble factors seems to facilitate adipogenesis. This observation is confirmed by up-regulation of lipid vacuoles formation and adipocyte-specific markers expression. Intact cytoskeleton and/or focal adhesion-mediated signaling appear to be the prerequisites for early adipogenic commitment of hMSC. However, following the initial biochemically-induced commitment, the cellular mechanics plays an increasingly important role in enhancing the stem cell differentiation efficiency. Our findings have significant implications for tissue engineering, reconstructive and cosmetic surgery, and other stem cell-based therapeutic applications.

3788-Pos
Mechanics of Molecular Bond Clusters between Elastic Media: Stochastic-Elastic Coupling in Cell-Matrix Adhesion
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Focal adhesions are clusters of specific receptor-ligand bonds that link an animal cell to an extracellular matrix. A capability to control focal adhesions, for which a quantitative description of the collective behavior of multiple molecular bonds is a critical step, is essential for tissue and cellular engineering. While the behavior of single molecular bonds is governed by statistical mechanics at small scale, continuum mechanics should be valid at large scale. How can this transition be modeled and can this tell us something about the mechanics of cell adhesion? Here we develop a stochastic-elasticity model of a periodic array of adhesion clusters between two dissimilar elastic media subjected to an inclined tensile stress, in which stochastic descriptions of molecular bonds and elastic descriptions of interfacial traction are unified in a single modeling framework. A fundamental scaling law of interfacial traction distribution is established to govern the transition between uniform and cracklike singular distributions of the interfacial traction within molecular bonds. Guided by this scaling law, we perform Monte Carlo simulations to investigate the effects of cluster size, cell/matrix modulus and loading direction on lifetime and strength of the adhesion clusters. The results show that intermediate adhesion size, stiff substrate, cytoskeleton stiffening, and low-angle pulling are factors that contribute to the stability of focal adhesions. The predictions of our model provide feasible explanations for a wide range of experimental observations and suggest possible mechanisms by which cells can modulate adhesion and deadhesion via cytoskeletal contractile machinery.

3789-Pos
Mapping Adhesion Turnover in Migrating Cells: An Image Cross-Correlation Study
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Cell migration requires the assembly and disassembly of adhesions, which provides a physical linkage between the actin cytoskeleton and the extracellular matrix (ECM). Adhesions are composed of many interacting molecules that also organize signals that regulate migration. We have previously used spatio-temporal image correlation spectroscopy (STICS) to observe the dynamics and nature of the linkage between actin and α_5-integrin as adhesions slide and disassemble. We now extend this study to probe the linkage using cross-correlation methods. STICCS analyzes the intensity fluctuations and calculates autocorrelation and cross-correlation functions in space and time to yield velocity vectors and diffusion coefficients for regions analyzed. This information is assembled into vector maps characterizing the movement of these proteins in different areas of the cell and at different times during the series acquisition. We applied a Fourier filter to remove contributions from static components to better resolve the dynamic protein populations and also carried out computer simulations to model moving boundaries and simulate edge effects to better